Synopsis

Bioinspired Synthesis of Metallic and Non-metallic Nanoparticles and Delving their Pharmacological, Biological, Cosmetic and Nanoswitching applications

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INTRODUCTION

The application of nanotechnology in functionalized and modified processes is promising for use in the delivery of drugs, bioimaging, diagnosis and therapy, genetic material sequencing, biosensors, active packaging, agriculture, and the environment (**Sharma *et al.*, 2021**). The prodigious application of nanotechnology inspired to carry out research in this field. Considering the importance of Gold nanoparticles (GNP), Silver nanoparticles (SNP), Zinc oxide nanoparticles (ZnONP) and reduced graphene oxide (RGO) are synthesized by adapting green synthetic routes (**Pissuwan *et al.*, 2020**).

Terminalia bellerica Roxb.(TB) *(Combretaceae)* universally called Bahera or Bastard myrobalan is a large tree which is commonly seen in moist Indian sub valleys. Almost all the plant parts are of high medicinal values (**Kumar and Khurana, 2018**). The whole fruit of TB aided reduced graphene oxide and its bacterial properties and anti-diabetic efficiency of TB is reported (**Sethumadhavan *et al.*, 2021**). Commercially available *Garcinia combogia* capsules suspended in water is utilized for nanoparticle synthesis. The capsule of *Garcinia combogia* is administrated for weight reduction. The fruit is also rich in Hydroxy Citric Acid (HCA) which is well known for hypo-lipidemic activity endorsing its use in weight management. There are limited studies on washed waters of cereals and pulses and free edge of *Corpus unguis*. The detailed study of these components is one of the objectives of the present study.

Still, multi microbe-resistant broad-spectrum antibiotics with lesser side effects are a big challenge for researchers as microbes are capable enough to obtain resistance to the drugs (**Abusaiba and Thualfakar, 2020**). The potential antimicrobial activity of GNP and SNP against gram-positive and gram-negative microbes was tested. The efficiency of the synthesized nanoparticles against clinical isolates of microbes (which might have already gained antibacterial resistance) has also been tested. Skin cancer is one of the most common carcinomas worldwide, and it is classified into two types: melanoma and non-melanoma. Pondering the need for a solution for anti-skin cancer agents, the present study focuses on determining the efficiency of GNP, SNP and washed waters of cereals and pulses against skin cancer cell lines.

Many prescribed drugs now in clinical use or advanced clinical studies have DNA as their pharmacological target. Considering the importance of DNA binding studies, the interaction

of synthesized GNP and SNP with ct-DNA was studied by a facile UV-visible spectrophotometric method. The *Allium cepa* toxicity assay is a popular and very sensitive technique for investigating chromosomal abnormalities induced by numerous chemical toxic substances. The *Allium cepa* toxicity assay is a popular and very sensitive technique for investigating chromosomal abnormalities induced by numerous chemical toxic substances. Because *A. cepa* has a small number of chromosomes (2n = 16), it is easy to examine the genetic alterations at the chromosomal level that occur after plants are exposed to harmful substances. This test has been used to look into the impact of toxins on the normalcy of cellular division. The test is an easy way to gain a thorough understanding of how harmful compounds, alone or in combination, cause chromosomal changes (**Sabeen *et al.*, 2020**). The present study also focuses on the toxicity determination of the synthesized best nanoparticles which have shown good pharmacological activity via, Allium cepa root tip assay. Over the last several decades, the use of sunscreens (also known as sun protectants) for protection against the detrimental effects of the sun's rays has increased. It is reported that ZnONP due to its excellent UV blocking properties are utilized in sun screen lotions. This study also utilizes the synthesized ZnONP in sunscreen lotions and aims to evaluate the sun protection factor by spectrophotometric methods.

Nowadays, the identification of certain compounds, molecules, or atoms at low concentrations is critically important, not only for our everyday comfort and safety but also for our increasingly contaminated environment. Carbon paste electrode is one of the oldest carbon-based electrodes, having been utilised in numerous electrochemical applications for several years (**Vancara *et al.*, 2009**). It is assumed that only melamine adulteration of food products is the only way to human exposure to melamine. A recent study reveals that melamine and its derivatives are found in indoor dust obtained from household materials made of melamine (**Zhu *et al.*, 2018**). An extensive study reveals that melamine-formaldehyde resin plates leach melamine when exposed to acidic foods at particular temperature conditions. The vast usage of melamine wares is because of its long durability and its ambient appearance with vibrant colours. A decade ago melamine emerged as a potent adulterant not only in food materials but also in animal feeds. Free cyanide and hydrogen cyanide (HCN) are both more dangerous forms of cyanide. Cyanide anion (CN^-) has the negative effect of creating complexes in the cell mitochondrion, interfering with a cell's ability to create energy and, as a result, leading to a chemical buildup in the bloodstream. Traditional cyanide testing procedures, such as titration,

distillation, chromatography, or potentiometry, are costly, time-consuming, and tedious. As a result, there is still a need to create more rigorous, low-cost, and labor-intensive cyanide testing procedures. A number of colorimetric detection techniques were developed in an effort to make anion quantitative detection and monitoring economical, simple, and easy to follow (**Gunnlaugsson *et al.*, 2006**). Among the potential chemicals Ammonia, Melamine and cyanide detection are essential as their presence in food as adulterant is high. Also the environmental contamination is relatively high. Recently, approximately 80% of NH_3 was produced for nitrogen-based fertiliser, with the remaining 20% produced for pharmaceuticals, cleaning products, explosives, and refrigeration using the Haber-Bosch process, which employs the reaction of hydrogen and nitrogen with an iron-based catalyst at high temperatures. NH_3 is commonly found in dairy and ice cream operations, wineries and breweries, petrochemical facilities, fruit and vegetable juice processing plants, and soft drink manufacturing facilities (**Kwak *et al.*, 2019**).In lieu of the aforesaid problems, this research focuses on detecting melamine, ammonia and cyanide by colorimetric and electrochemical methods by fabricating reduced graphene oxide, GNP- based carbon paste electrodes.

Objectives of the study

Primary objectives

- To synthesize Metallic nanoparticles, such as Gold, Silver, and Zinc oxide, as well as Non-metallic nanoparticles, such as reduced graphene oxide, using plant extracts (aqueous), cereals, pulses washed water, and Human nail washings (aqueous)
- to study the nature of nanoparticles by analytical characterization techniques and classify their applications;
- to explore selected biological, pharmacological, cosmetic, and colorimetric/electrochemical/analytical sensing applications of the synthesized nanoparticles.

Objectives in detail

i. To prepare the extracts (aqueous) of *Terminalia bellirica* dry fruit parts and *Garcinia cambogia* capsules by steam bath method and preparation of cereals and pulses washed water and *Corpus unguis* free edge is sonicated in water to obtain the washed solution

ii. To screen the components of the reducing agents by phytochemical analysis (aqueous extracts), nutritional analysis (cereals and pulses washed water)

iii. To synthesize Gold, Silver and Zinc oxide and reduced graphene oxide nanoparticles using the prepared reducing agents

iv. To optimize the synthesis condition of metallic nanoparticles by different methods of synthesis viz, room temperature, sonication, solar irradiation and microwave heating methods and the effect of reducing agents upon synthesis method and time of formation of nanoparticles

v. To characterize the synthesized nanoparticles by analytical techniques

vi. To evaluate the antibacterial activity, anti-cancer activity of gold and silver nanoparticles against skin cancer cell lines and to develop anti-bacterial earphone buds

vii. To formulate Skin lotions incorporating Zinc oxide nanoparticles and human nail washings aided gold nanoparticles and their Sun protection factor determination

viii. To peruse the colorimetric and electrochemical sensing action of synthesized Gold nanoparticles to sense CN^- and NH_3

ix. To detect Melamine leached from Melamine tableware by FTIR fingerprinting analysis and toxicity assessment by comet assay and to impregnate reduced graphene oxide in carbon paste and electrode fabrication for the detection of melamine by electrochemical analysis.

MATERIALS AND METHODS

Materials used

Terminalia bellirica dry fruit was collected from the local herbal shop in Palakkad district, Kerala, India. Various Indian Rice grains, Toor dhal, and Chana, were collected from local shops in the Coimbatore district. Rice varieties from Thailand, Indonesia and Boston were also utilized in this study. *Garcinia combogia* capsules were purchased from the local shop in Coimbatore. Aqueous extracts were subjected to phytochemical screening. Cereals and pulses of washed water were analyzed for nutrient analysis and elemental analysis (EDS).

Synthesis of Metallic and Non-metallic nanoparticles

Gold nanoparticles were prepared via, room temperature, ultrasonic bath, solar irradiation and Microwave oven heating method. Silver nanoparticles by solar irradiation method, ZnONP by precipitation method and RGO by reflux method.

Characterization of Metallic and Non-metallic nanoparticles

Metallic and non-metallic nanoparticles were characterized using UV-visible, FT-IR, XRD, Zeta potential, TGA, Raman, FE-SEM and EDS spectroscopic analysis.

Pharmacological and Biological activities

Synthesized GNP and SNP were tested for anti-bacterial activity against clinical isolates *Acinetobacter pneumonia, Bacillus subtilis, Enterococcus faecalis, Bacillus subtilis, K. pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*. Best SNP samples are coated over earphone buds and developed antibacterial earphone buds. Anti-skin cancer activity of GNP and SNP against A375 cell lines was carried out. CT-DNA binding interaction of GNP and SNP was studied by spectrophotometric analysis. *Allium cepa* root tip toxicity assay was carried out for the best samples which possess biological activity.

Lotion formulations incorporating GNP and ZnONP

GNP synthesized from Human nail waste water and ZnONP prepared from raw rice-washed water was incorporated. The quality of the samples was assured by the physico-chemical analysis and anti-microbial analysis against *Candia albicans* and *Staphylococcus aureus* which causes skin infections.

Nanoswitching applications of GNP and RGO

The switching of TB-aided GNP when exposed to Ammonia a common food adulterant were studied via colorimetric method. *Garcinia combogia* aided GNP incorporated in carbon paste electrodes and electrochemical parameters were noted and GNP switching behavior was studied when exposed to cyanide solution. The switching behaviour of RGO incorporated in carbon paste electrode and its behaviour towards melamine sensing was also studied. Melamine

plates incubated with common Indian cuisines and leaching of melamine by FT-IR fingerprinting was done and genotoxic studies by comet assay were also studied.

RESULTS AND DISCUSSION

Results of Phytochemical and Nutrient Analysis

The phytochemical analysis reveals the presence of flavonoids, phenols and Tannins in the sample. The presence of Vitamin B6, carbohydrates, proteins,and fats in cereals and pulses washed water. No toxic elements were present in the cereals and pulses washed water, which was confirmed by the EDS analysis.

Results of Nanoparticle synthesis

The nanoparticle prepared via the Microwave oven method requires only 5s to 90s for the formation of GNP and in the room temperature method same samples require nearly 9h. The colours of the GNP obtained are pink, purple,and violet. The SNP synthesis by solar irradiation method requires 5mins to 30mins for the change in colour to brown. The white precipitate formed ensures the formation of ZnONP. The reduction of graphene oxide is ensured by the colour change of the black solution.

Results of characterization of nanoparticles

The surface plasmon resonance at 500-600nm (GNP), 380-450nm (SNP), 300-400nm (ZnONP) ensures the formation of nanoparticles. The C=O, C-metal, C=C, O-H, N-H peaks were observed in FT-IR spectra. XRD shows the crystalline/amorphous nature and crystallite size of nanoparticles. From Zeta analysis, the stability of GNP and SNP was ensured. TGA of RGO shows the thermal stability upto 1000^{o}C. FESEM analysis shows spherical and clustered particles.

Results of pharmacological and biological activity of nanoparticles

The GNP and SNP show inhibition towards both gram-negative and gram-positive pathogens. TB-aided SNP show selective inhibition against *S.aureus*. The SNP-coated earphone buds also shows bacterial resistance. Compared to SNP, the IC_{50} value of GNP is lesser against

A375 cell lines. GNP and SNP shows intercalation mode towards CT-DNA binding. The nanoparticle-incubated *Allium cepa* root tip doesn't show any chromosomal abrasion.

Results of Physico-chemical analysis of Lotion formulation

SPF of GNP incorporated lotion was 4.3394 and ZnONP was 18.16 in the UV B region by spectrophotometric method. The samples show good moisturizing effects too. The pH of the samples was 5-6. The samples show good spreadability compared to standard sunscreen lotion.

Results of Nanoswitching applications of Nanoparticles

The TBGNP shows the visual colour change switching upto 5000ppm of NH_3. It was also observed that nanoswitching is due to ammonia gas. The carbon paste with GNP was able to detect CN^- upto 25mM of CN^- in tap water. RGO-incorporated carbon paste electrodes were able to detect melamine (20μM) spiked in raw milk. FT-IR fingerprint matches with food samples incubated in melamine plates. The samples show genotoxicity due to melamine leaching. The low-cost electrode using corn sticks also shows conductivity in electrochemical analysis

CONCLUSION

The efficient synthesis of GNP, SNP, ZnONP and RGO was possible from the green reducing agents. The potential metabolite in the reducing agents helps in the reduction process. The synthesized nanoparticle shows significant antibacterial activity against clinically isolated gram-positive and gram-negative metabolites, toxicity towards A375 skin cancer cell lines, DNA intercalation and no chromosomal abrasions. The lotion formulations prepared show sun protection activity compared to the commercially available sunscreen lotions. The synthesized nanoparticles show nanoswitching properties when exposed to food adulterants such as Ammonia, Melamine and Cyanide. The nanoparticle behaves as self-powered nano switches.

PUBLICATIONS

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